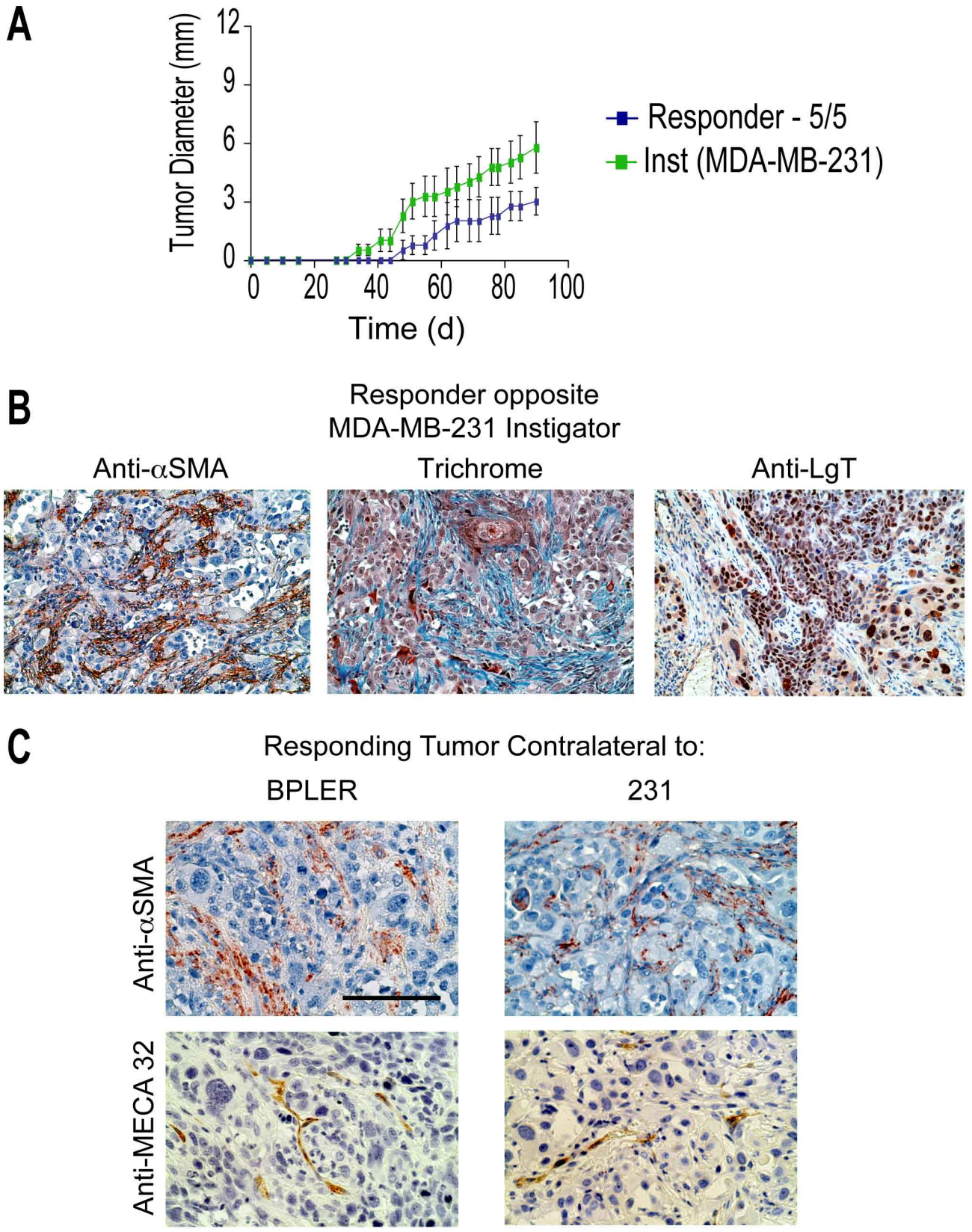
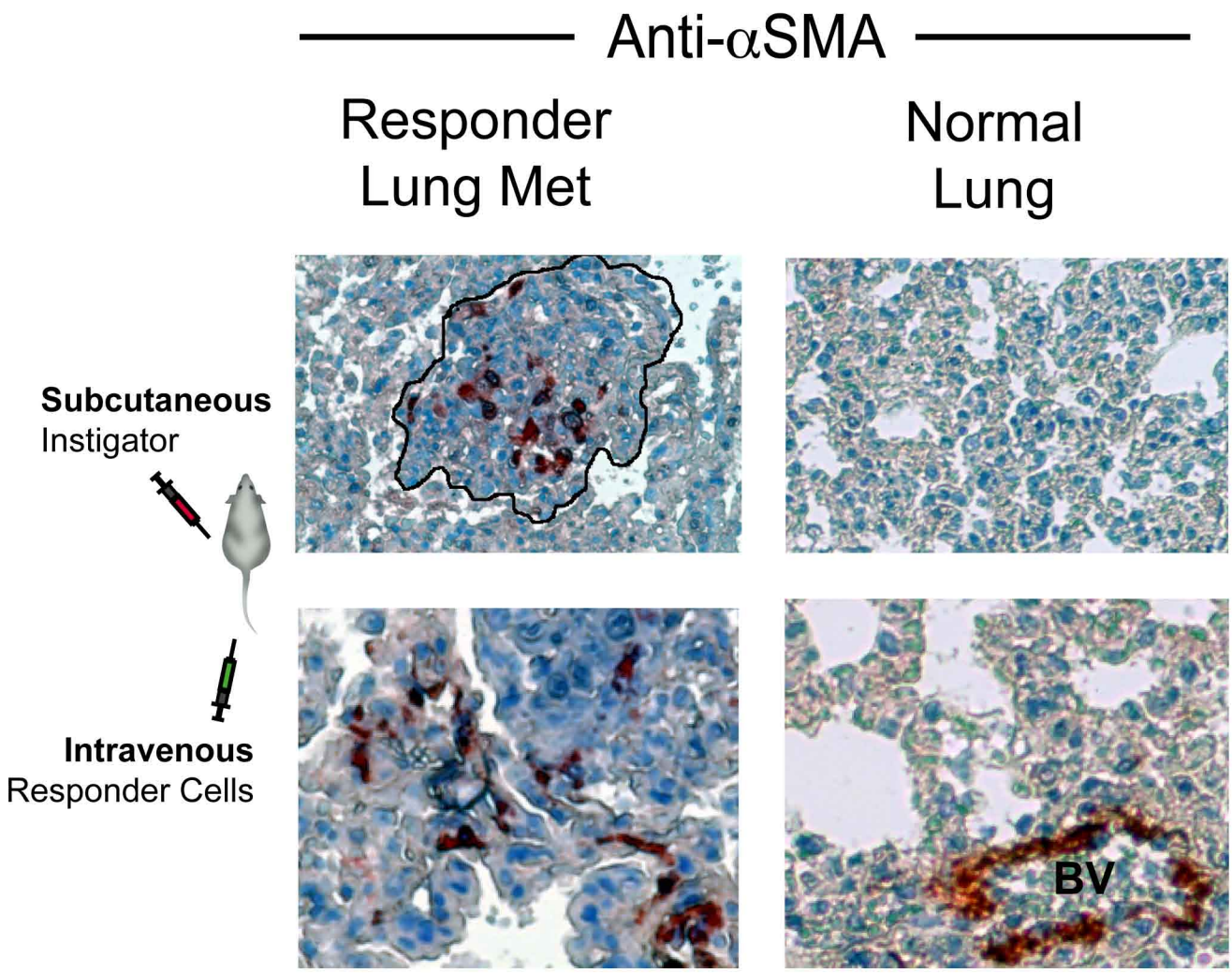


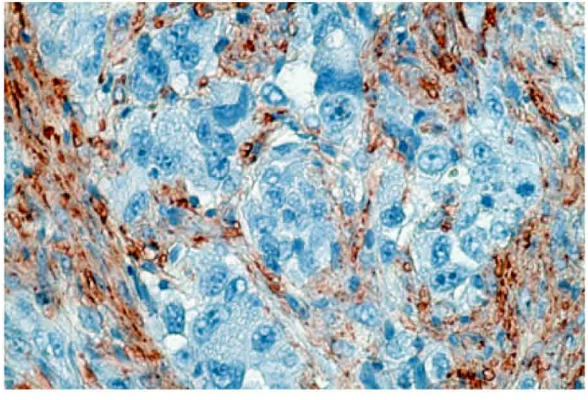
Supplementary Figure 1. Responding tumor growth kinetics and histopathology mediated by instigating MDA-MB-231 breast cancer cells. (A) Growth kinetics of instigating and responding tumors implanted bilaterally in Nude mice(n=5). **(B)** Histopathology of resulting responding tumors when injected contralaterally to instigating MDA-MB-231 tumors. Left: Alpha smooth muscle actin (αSMA) staining of myofibroblasts and pericytes (brown) and hematoxylin counterstaining of nuclei (blue). Center: Masson's Trichrome staining for collagen (blue) and cell nuclei (dark pink). Right: Staining for SV40 large T antigen (LgT) to identify responding tumor cells. Magnification: 20x. **(C)** Serial sections of responding tumors growing contralaterally to BPLER instigator tumors (left) or MDA-MB-231 instigating tumors (right) stained for αSMA (brown, top) or mouse endothelial cell antigen (MECA32, brown, bottom); nuclei counterstained with hematoxylin (blue). Images indicate that αSMA+ cells did not exclusively associate with MECA32+ cells. Scale bar = 100μm.



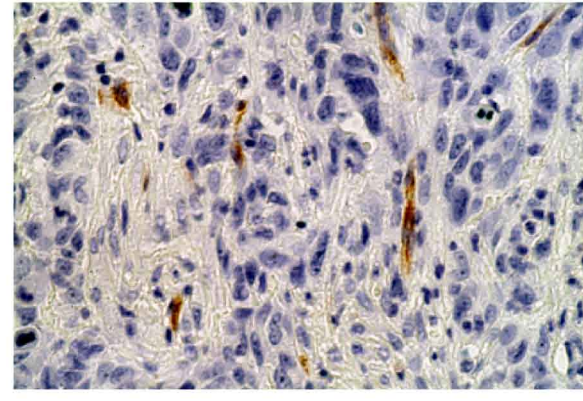
Supplementary Figure 2. Instigating tumors facilitate stromal desmoplasia in responding lung tumor colonies. Cartoon shows schematic of the systemic instigation of lung metastases. Images represent responder cell colonies formed in the lungs of mice bearing subcutaneous instigating tumors (left panels) compared with normal lungs from tumor-free mice (right panels). Lung tissues were stained for αSMA (brown) and nuclei counterstained with hematoxylin (blue). Outline (top left) shows a responding tumor in the lung. Bottom right indicates staining of a normal blood vessel (BV) in the lung.

Responder Cells + Instigator BMCs

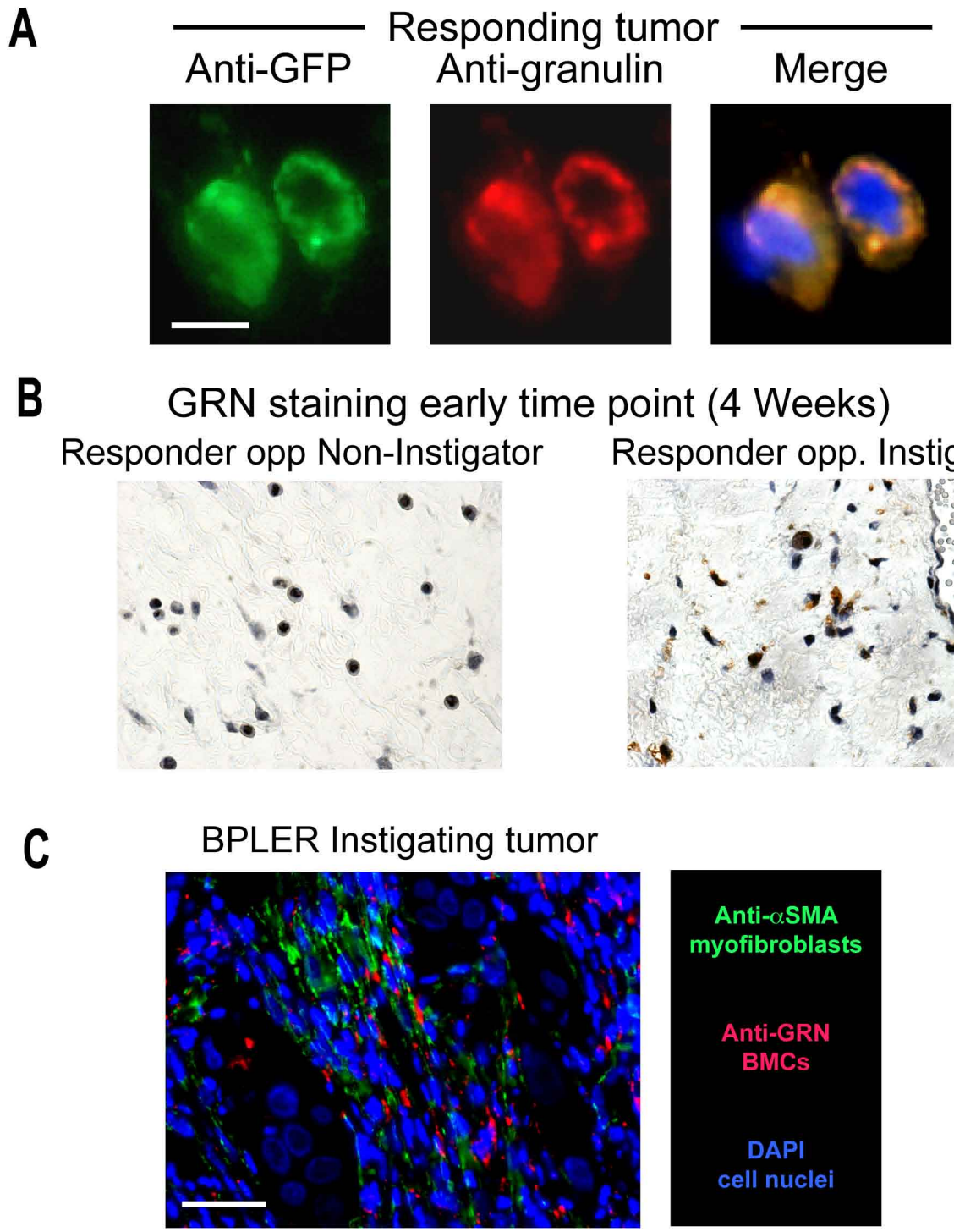
Anti-αSMA



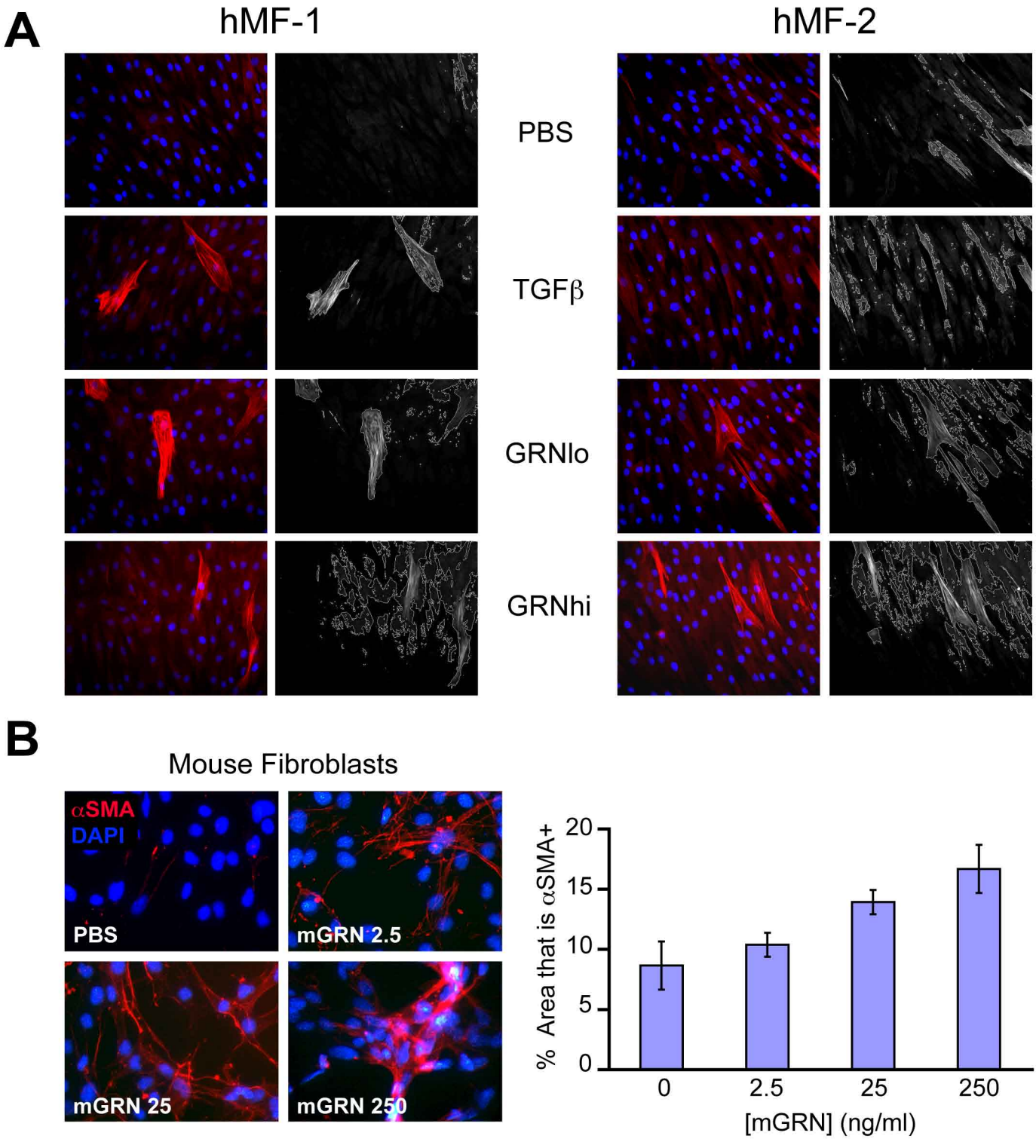
Anti-MECA 32



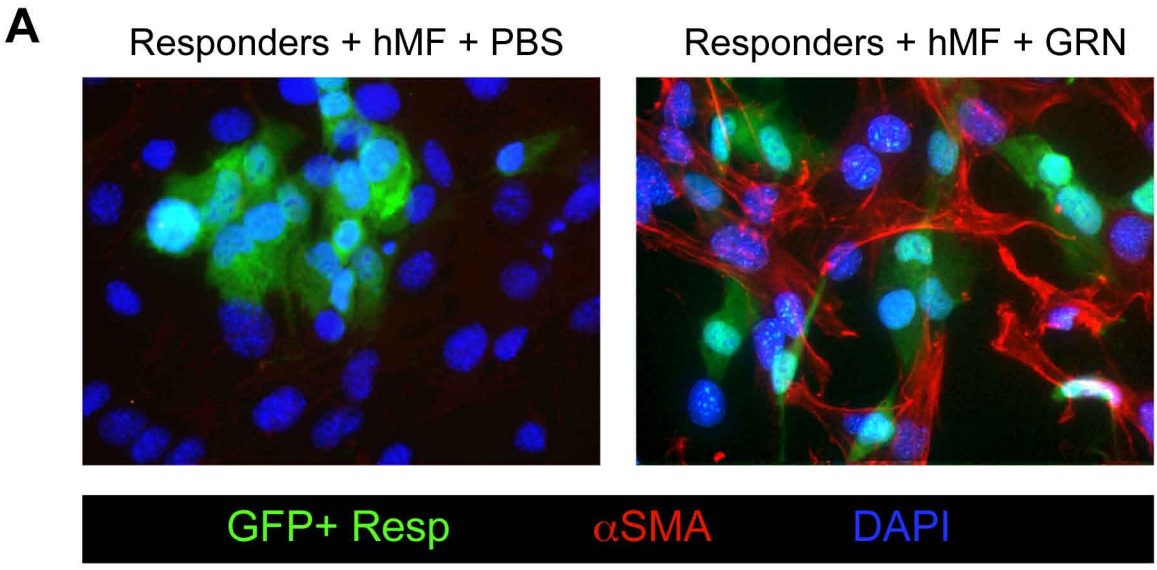
Supplementary Figure 3.Instigating BMCs enhance myofibroblast-rich desmoplastic stroma in responding tumors. Photomicrographs represent growing responding tumors resulting from admixture of responder cells with BMCs from instigator-bearing mice. Serial sections were stained for αSMA (brown, left) or MECA32 (brown, right) and hematoxylin-stained nuclei (blue).

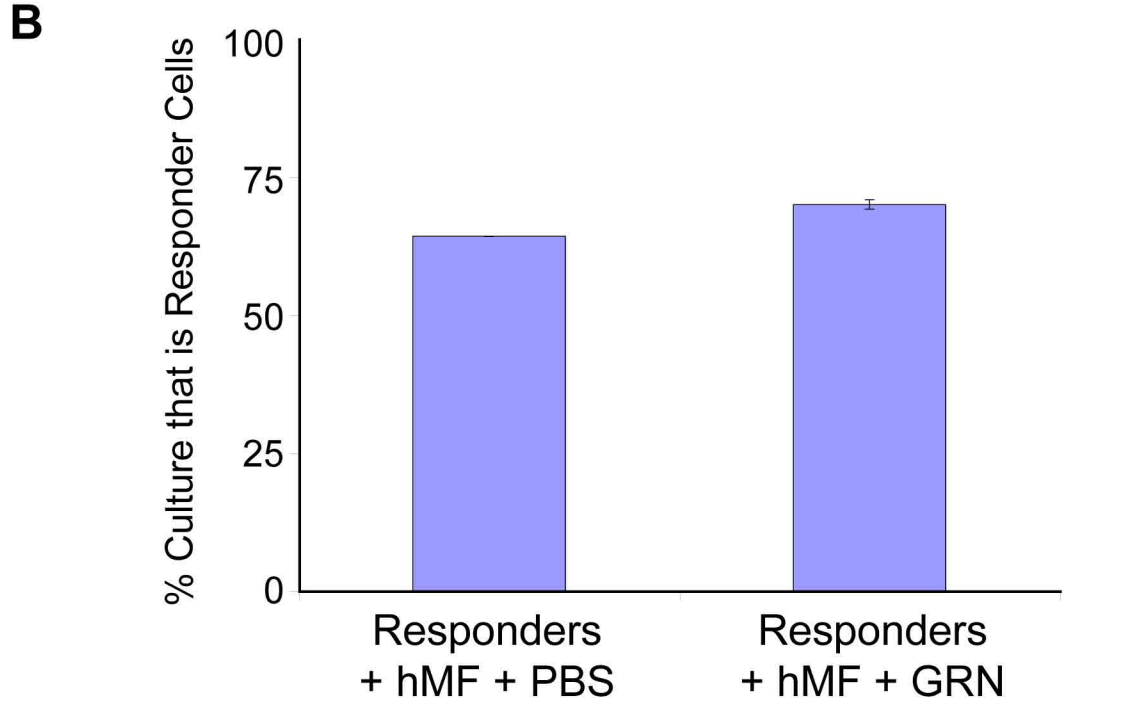


Supplementary Figure 4. GRN+ hematopoietic cells in the tumor stroma are BM-derived but do not give rise directly to tumor myofibroblasts. (A) Immunofluorescent images of responding tumors growing contralaterally to instigating tumors for 4 weeks: staining for GFP+ BM-derived cells (green) and Granulin (red). Analysis indicated that the majority of GRN+ cells in the responding tumor stroma were BM-derived (yellow). Scale bar=12.5μm. **(B)** Staining for GRN (brown) and cell nuclei (blue) in responder plugs recovered opposite non-instigating tumors (left) or instigating tumors (right) 4 weeks after injection. **(C)** Merged immunofluorescent image of a BPLER instigating tumor after 12 weeks of in vivo growth. GRN+ BMCs (red) were closely associated with stromal myofibrobalsts (green); cell nuclei were stained with DAPI (blue). Scale bar = 50μm.

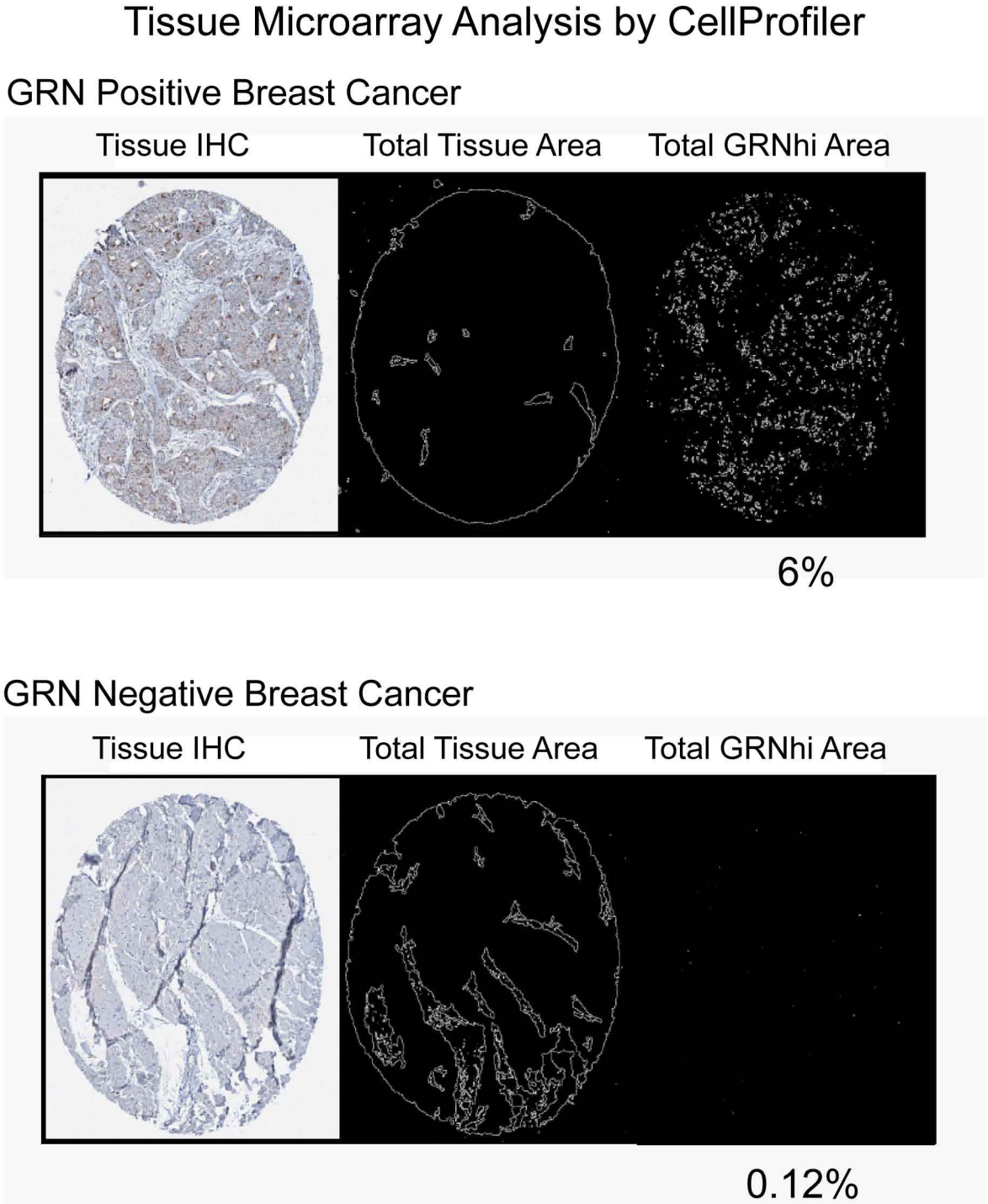


Supplementary Figure 5. GRN induces αSMA expression in human mammary and primary murine fibroblasts in a dose-dependent fashion. (A) Representative images and CellProfiler outlines used for quantification of αSMA (red) expression in two different preparations of cultured primary human mammary fibroblasts (hMF-1 and hMF-2) treated as indicated; cell nuclei were stained with DAPI (blue). This figure corresponds with data represented in Fig 6. **(B)** Cultured primary mouse fibroblasts, CH310T1/2, were treated with control PBS or indicated doses of recombinant mouse GRN and stained for αSMA (red) and DAPI (blue). Graph represents CellProfiler quantification of the percent total image area covered by αSMA+ staining (see Methods) at indicated doses of GRN treatment (n=10 images per group).



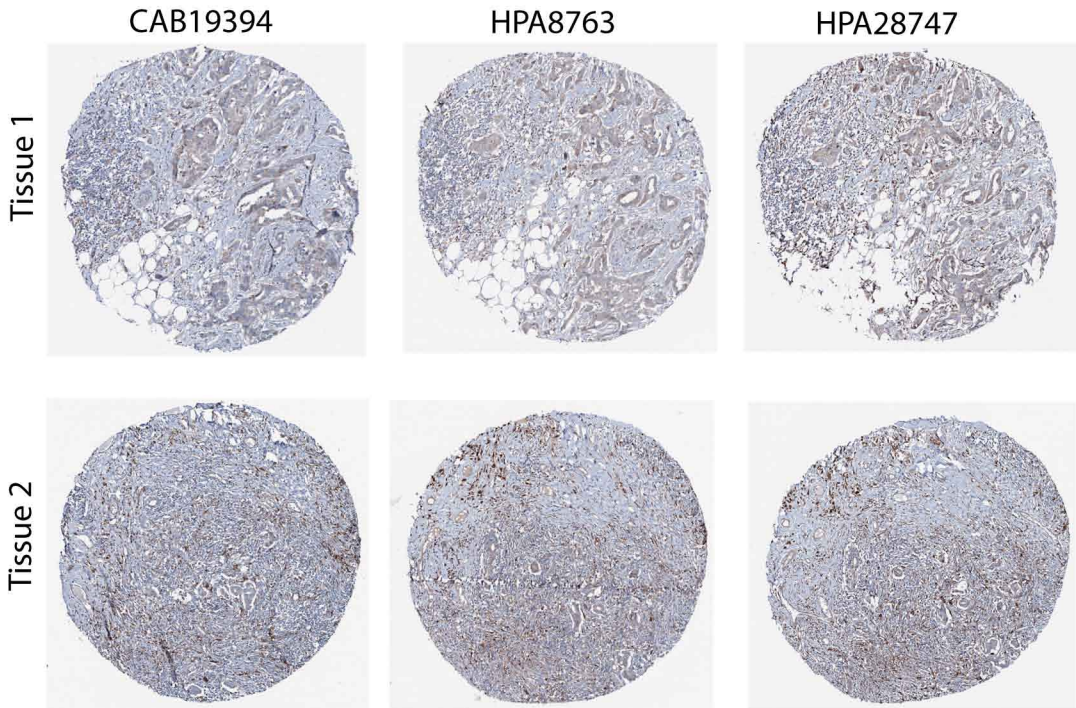


Supplementary Figure 6. GRN-activated fibroblasts do not affect the proliferation of responder cells *in vitro.* (A) Images show GFP+ responding tumor cells (green) co-cultured with human mammary fibroblasts (hMF) at a 1:1 ratio and treated for 6 days with either PBS (left) or GRN (right). Cultures were stained with anti-αSMA to visualize activated fibroblasts (red) and nuclei stained with DAPI (blue). **(B)** Graph represents the contribution of GFP+ responder cells to the indicated cultures after 6 days, as determined by flow cytometry (n=6 per group). At the start of the experiment (d0), responder cells were cultured with fibroblasts at a ratio of 1:1.



Supplementary Figure 7. CellProfiler analysis of GRN staining of human breast tumor tissue microarray. Examples of a GRN-positive (top) and a GRN-negative (bottom) tumor stained for GRN using antibody HPA028747. Human tissues were stained for GRN (brown, left panels)and outlines of total tissue area (center panels) and high-intensity GRN staining (right panels) were calculated by CellProfiler software. Numbers indicate percentage of total tissue image area occupied by high-intensity GRN expression.

A



B

	CAB19394			HPA8763		
	Correlation coefficiency	Sig (2-tailed)	N	Correlation coefficiency	Sig (2-tailed)	N
Age	0.129	0.137	134	0.094	0.281	132
Tumor size	0.180	0.037	134	0.301	0.000	132
Grade	0.150	0.083	134	0.414	0.000	132
Nodal Stage	0.027	0.771	122	0.112	0.227	119
Histological subtype	-0.193	0.026	134	-0.275	0.001	132
Her 2 status	0.021	0.815	131	0.166	0.06	129
Her2 subtype	0.106	0.23	130	0.1	0.259	128
Manual ER status	0.151	0.081	134	-0.251	0.004	132
Manual PR status	0.048	0.579	134	-0.294	0.001	132
Triple negative	0.257	0.003	130	0.211	0.017	128
Molecular subtype	0.132	0.129	134	0.120	0.171	132
Luminal B	0.006	0.947	130	0.067	0.452	128
Luminal A	-0.191	0.032	127	-0.238	0.007	125
Basal	0.257	0.003	130	0.211	0.017	128
KI673g	0.124	0.184	117	0.352	0.000	115

Supplementary Figure 8. GRN staining and clinicopathological characteristics of 144 cases of breast cancer. (A) Representative images of breast tumor tissues stained with anti-GRN antibodies: CAB19394, HPA8763, and HPA28747. **(B)** Correlation of GRN staining (antibodies CAB19394 and HPA8763) with indicated clinicopathologic features in the breast cancer patient cohort used to construct tissue microarrays. Statistically significant positive correlations are represented in red typeface, negative correlations in blue, and no significant correlation in black.

Age	
Mean	65
Median	64
(Range)	34-97
Menopausal status	
Pre	20
Peri	3
Post	118
Missing	3
Type of surgery	
Breast conserving	63
Mastectomy	81
Multifocal disease	
No	107
Yes	37
Tumour size (mm)	
Mean	26
Median	20
Range	6-145
<=20 mm (T1)	73
>20 mm(T2+)	71
Examined nodes	
Mean	11.40
Median	12.00
Range	0-25
Nodal Stage	
0	73
1-3	35
>=4	21
Missing	15
NHG	
I	22
II	64
III	58

Endocrine treatment	
No	47
Yes	96
Missing	1
Tamoxifen	67
AI	3
Tam + AI	25
Missing	49
Chemotherapy	
No	113
Yes	30
Missing	1
FEC	26
CMF	3
Taxol	2
Neoadjuvant	2
Recurrence	
No	115
Yes	29
Follow-up	
Mean	5.78
Median	6.55
Range	0.33-7.55
Vital status	
Dead	103
Alive	41
Dead from breast cancer	122
Histological subtype	
Mixed ductal and lobular	3
Ductal	104
Lubular	27
Tubular	7
Medullary	3

ER status	
Negative	19
Positive	125
PgR status	
Negative	44
Positive	100
HER2 IHC	
0	88
1	30
2	12
3	9
Missing	5
Molecular subtype	
Normal	7
Luminal A	8
Luminal B	109
HER2	5
Basal	15

Table S1. Clinicopathologic characteristics of breast cancer patient cohort used to construct tissue microarray.